

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a sequence selected from the group consisting of
5 SEQ ID NOS:79-98, or a fragment, region, or *cis* element of said sequence thereof, said
isolated nucleic acid being capable of regulating transcription of an operably linked DNA
sequence.
2. The isolated nucleic acid of claim 1 wherein the isolated nucleic acid is a promoter.
3. The isolated nucleic acid of claim 2 wherein the promoter is a hybrid promoter.
- 10 4. The isolated nucleic acid of claim 3 wherein said isolated nucleic acid confers enhanced
expression of operably linked genes in male reproductive tissues.
5. The isolated nucleic acid of claim 4 wherein said isolated nucleic acid confers enhanced
expression of operably linked genes in anthers.
6. The isolated nucleic acid of claim 5 wherein said isolated nucleic acid confers enhanced
15 expression of operably linked genes in wheat anthers.
7. The isolated nucleic acid of claim 4 further comprising a minimal promoter.
8. The isolated nucleic acid of claim 7 wherein the minimal promoter is selected from the
group consisting of a minimal CaMV and a rice actin promoter.
9. The isolated nucleic acid of claim 8 wherein the minimal promoter is a minimal CaMV
20 35S promoter.
10. A promoter comprising a nucleic acid sequence selected from the group consisting of
SEQ ID NOS: 79-98 and fragments thereof.
11. The promoter of claim 10 wherein said promoter confers enhanced expression of
operably linked genes in male reproductive tissues.
- 25 12. The promoter of claim 11 wherein said promoter confers enhanced expression of
operably linked genes in anthers.
13. The promoter of claim 12 wherein said promoter confers enhanced expression of
operably linked genes in wheat anthers.
14. A cell comprising a DNA construct comprising an isolated nucleic acid sequence selected
30 from the group consisting of SEQ ID NOS:79-98 or a fragment, region, or *cis* element of

said sequence thereof, and operably linked to said nucleic acid sequence, a transcribable DNA sequence and a 3' non-translated region.

15. A transgenic plant comprising a DNA construct comprising an isolated nucleic acid sequence selected from the group consisting of SEQ ID NOS:79-98 or a fragment,
5 region, or *cis* element of said sequence thereof, and operably linked to said nucleic acid sequence, a transcribable DNA sequence and a 3' non-translated region.

16. A method of regulating transcription of a DNA sequence comprising operably linking the DNA sequence to a promoter comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS:79-98.

10 17. The method of claim 16 comprising operably linking the DNA sequence to a hybrid promoter comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS:79-98.

18. The method of claim 16 wherein operably linking the nucleic acid sequence selected from the group consisting of SEQ ID NOS:79-98 or fragment thereof to the promoter
15 confers enhanced expression of operably linked genes in male reproductive tissues.

19. The method of claim 18 wherein said male reproductive tissues comprise monocot or dicot male reproductive tissues.

20. The method of claim 19 wherein said male reproductive tissues comprise anthers.

21. The method of claim 20 wherein said male reproductive tissues comprise wheat anthers.

20 22. The method of claim 16 comprising operably linking a minimal promoter to the nucleic acid sequences selected from the group consisting of SEQ ID NOS:79-98 or fragment, region, or *cis* element thereof.

23. A method of making a transgenic plant comprising introducing into a cell of a plant a DNA construct comprising: (i) a promoter comprising a nucleic acid sequence selected
25 from the group consisting of SEQ ID NOS:79-98 or a fragment, region or *cis* element thereof, and, operably linked to the promoter, (ii) a transcribable DNA sequence and (iii) a 3' non-translated region.

24. A method of isolating at least two 5' regulatory sequences that confer enhanced expression of operably linked genes in male reproductive tissues from a plant
30 comprising:

- (i) evaluating a collection of nucleic acid sequences of ESTs derived from at least one cDNA library prepared from a plant cell type of interest;
- (ii) comparing EST sequences from at least one target plant cDNA library and at least one non-target cDNA libraries of ESTs from a different plant cell type;
- 5 (iii) subtracting common EST sequences found in both target and non-target libraries;
- (iv) designing gene specific primers from the remaining EST sequences after said subtraction; and
- (v) isolating the corresponding 5' flanking and regulatory sequences from a genomic library prepared from the target plant comprising the use of said primers.

- 10 25. The method of claim 24 wherein said male reproductive tissues are from monocot or dicot plants.
- 26. The method of claim 25 wherein said male reproductive tissues comprise anthers.
- 27. The method of claim 26 wherein said male reproductive tissues comprise wheat anthers.

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